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Polymorphisms in the human CYP3A4, CYP3A7 and hPXR genes and their use in diagnostic and therapeutic applications

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**Title of the invention**

**Polymorphisms in the human CYP3A4, CYP3A7 and hPXR genes and their use in diagnostic and therapeutic applications**

**Field of the invention**

The present invention relates generally to means and methods of diagnosing and treating the phenotypic spectrum as well as the overlapping clinical characteristics with several forms of inherited abnormal expression and/or function of the cytochrome P-450 (CYP)3A4 and CYP3A7 and the human pregnane X receptor (hPXR) genes. In particular, the present invention relates to polynucleotides of molecular variant CYP3A4, CYP3A7 and hPXR genes which, for example, are associated with abnormal drug response or individual predisposition to several common cancers caused by environmental carcinogens, and to vectors comprising such polynucleotides. Furthermore, the present invention relates to host cells comprising such polynucleotides or vectors and their use for the production of variant CYP3A4, CYP3A7 and hPXR proteins. In addition, the present invention relates to variant CYP3A4, CYP3A7 and hPXR proteins and antibodies specifically recognizing such proteins. The present invention also concerns transgenic non-human animals comprising the above-described polynucleotide or vectors. Moreover, the present invention relates to methods for identifying and obtaining drug candidates and inhibitors for therapy of disorders related to the malfunction of the CYP3A4, CYP3A7 and hPXR genes as well as to methods of diagnosing the status of such disorders. The present invention furthermore provides pharmaceutical and diagnostic compositions comprising the above-described polynucleotides, vectors, proteins, antibodies, and drugs and inhibitors obtainable by the above-described method. Said compositions are particularly useful for diagnosing and

CYP3A gene family in the microsomes. Furthermore, Paolini et al. (7) found significant increases in CYP3A in the lungs of rats treated with high doses of beta-carotene. Consequently, it was proposed that correspondingly high levels of CYP3A4 in humans would predispose an individual to cancer risk from the bioactivated tobacco-smoke procarcinogens, thus explaining the cocarcinogenic effect of beta-carotene in smokers. All this implies that individual variation in the CYP3A4 activity could influence the efficacy of a variety of drug therapies as well as the individual predisposition to several major cancers caused by environmental carcinogens.

A considerable variation in the CYP3A4 content and catalytic activity has been, indeed, described in the general population. For example, the metabolic clearance of the gene substrates exhibits a unimodal distribution with up to 20-fold interindividual variability. The activities of the CYP3A4 protein in liver biopsies vary up to 30-fold (8). Furthermore, many common drugs alter the expression levels of the gene (induction or repression) and the extent of this phenomenon is individually variable. The inducers of CYP3A4 expression include commonly used drugs such as the glucocorticoid dexamethasone, the antibiotic rifampicin, and the antimycotic clotrimazole. The inducibility of CYP3A4 expression, combined with the diverse range of substrates, creates a potential for potentially harmful drug interactions involving this isozyme in patients undergoing therapies with multiple drugs.

CYP3A3 is a very closely related isoform to CYP3A4 (>more than 98% cDNA sequence similarity), but it is not known whether this reflects a separate gene product or an allelic variant. By contrast, CYP3A5 is a gene distinct from CYP3A4 and it is expressed polymorphically both in the adult and fetal liver and in the kidney and intestine. In adult Caucasians, the mRNA and the protein were detected in the liver of 10 to 30% of samples, while the protein was detected in the kidney and intestine of 70% of subjects (Ref. (9) and references therein). A point mutation described in the CYP3A5 gene which possibly results in the synthesis of an unstable protein, may account for the polymorphic expression of this enzyme (9). CYP3A7 is the third functional CYP3A isoform. CYP3A7 was originally isolated from a fetal liver but it was subsequently found in 54% of adult livers (10).

Tests to estimate the inducibility and the activity of CYP3A isozymes in an individual patient would be of obvious relevance for the optimization of therapies with drugs which

these genes. Alternatively, such polymorphisms could serve as markers for nearby, unidentified polymorphisms. This effect is known as linkage, i.e. defined polymorphisms serve as markers for phenotypes that they are not causative for.

A major breakthrough in the understanding of the CYP3A expression and inducibility took place in 1998 when three research groups independently showed that the expression of CYP3A4 is regulated by a member of the orphan nuclear receptor family termed PXR (pregnane X receptor), or PAR (22-24). Upon treatment with inducers of CYP3A4, PXR binds to the rifampicin/dexamethasone response element in the CYP3A4 promoter as a heterodimer with the 9-cis retinoic acid receptor (RXR). Northern blot analysis detected most abundant expression of hPXR in liver, colon, and small intestine, i.e. in the major organs expressing CYP3A4. The available evidence suggests that human PXR serves as a key transcriptional regulator of the CYP3A4 gene. A recent report describes the induction of CYP3A7 mediated by PXR suggesting that all members of the family may be regulated by this common transcriptional activator (25).

It is clear that naturally occurring mutations, if they exist can have effects on drug metabolism and efficacy of therapies with drugs, in particular in cancer treatment. It is unknown, however, how many of such variations exist, and with what frequency and at what positions in the human CYP3A4, CYP3A7 and hPXR genes.

Accordingly, means and methods for diagnosing and treating a variety of forms of individual drug intolerance and inefficacy of drug therapy which result from CYP3A4, CYP3A7 and/or hPXR gene polymorphisms that interfere e.g., with chemotherapeutic treatment of diseases, in particular cancer, was hitherto not available but are nevertheless highly desirable.

Thus, the technical problem of the present invention is to comply with the needs described above.

The solution to this technical problem is achieved by providing the embodiments characterized in the claims.

### Description of the invention

The finding and characterization of variations in the CYP3A4, CYP3A7 and hPXR genes, and diagnostic tests for the discrimination of different CYP3A4, CYP3A7 and hPXR alleles in human individuals provide a very potent tool for improving the therapy of diseases with drugs that are targets of the CYP3A4 or CYP3A7 gene product, and whose metabolism is therefore dependent on CYP3A4 or CYP3A7. The diagnosis of the individual allelic CYP3A4, CYP3A7 and hPXR status permits a more focused therapy, e.g., by opening the possibility to apply individual dose regimens of drugs. It may also be useful as prognostic tool for therapy outcome. Furthermore, diagnostic tests to genotype CYP3A4, CYP3A7 and hPXR, and novel CYP3A4, CYP3A7 and hPXR variants, will not only improve therapy with established drugs and help to correlate genotypes with drug activity or side effects. These tests and sequences also provide reagents for the development of novel inhibitors that specifically modulate the activity of the individual types of CYP3A4, CYP3A7 and hPXR. Expression in yeast, for example, of three allelic cDNAs encoding human liver CYP3A4 and methods for testing the binding properties and catalytic activities of their gene products have been described in (13).

Thus, the present invention provides a novel way to exploit molecular biology and pharmacological research for drug therapy while bypassing their potential detrimental effects which are due to expression of variant CYP3A4, CYP3A7 and hPXR genes.

Accordingly, the invention relates to a polynucleotide selected from the group consisting of:

- (a) polynucleotides comprising a nucleotide sequence encoding the amino acid sequence encodable by a nucleotide sequence of SEQ ID NO: 86, 94 and/or 98;
- (b) polynucleotides comprising a nucleotide sequence encoding a molecular variant of the cytochrome CYP3A4 protein by way of at least one amino acid deletion, addition and/or substitution at an amino acid position corresponding amino acid residue Gly56 in exon 3 of the CYP3A4 gene;
- (c) polynucleotides comprising a nucleotide sequence encoding a molecular variant of the cytochrome CYP3A7 protein by way of at least one amino acid deletion, addition



CYP3A7 and hPXR gene alleles that are present in the individual which provided the blood sample. The sequence analysis was carried out by PCR amplification of relevant regions of the CYP3A4, CYP3A7 and hPXR gene, subsequent purification of the PCR products, followed by automated DNA sequencing with established methods (ABI dyeterminator cycle sequencing).

One important parameter that had to be considered in the attempt to determine the individual CYP3A4, CYP3A7 and/or hPXR genotype and identify novel CYP3A4, CYP3A7 or hPXR variants by direct DNA-sequencing of PCR-products from human blood genomic DNA is the fact that each human harbors (usually, with very few abnormal exceptions) two gene copies of each autosomal gene (diploidy). Because of that, great care had to be taken in the evaluation of the sequences to be able to identify unambiguously not only homozygous sequence variations but also heterozygous variations. The details of the different steps in the identification and characterization of novel CYP3A4, CYP3A7 and hPXR gene polymorphisms (homozygous and heterozygous) are described in the examples below.

The mutations in the CYP3A4, CYP3A7 and hPXR genes detected in accordance with the present invention are illustrated in Figure 5 and 6, respectively (indicated by an arrow). The methods of the mutation analysis followed standard protocols and are described in detail in the examples. In general such methods to be used in accordance with the present invention for evaluating the phenotypic spectrum as well as the overlapping clinical characteristics with other forms of drug metabolization and altered tolerance to drugs in patients with mutations in the CYP3A4, CYP3A7 or hPXR gene encompass for example haplotype analysis, single-strand conformation polymorphism analysis (SSCA), PCR and direct sequencing. On the basis of thorough clinical characterization of many patients the phenotypes can then be correlated to these mutations as well as to mutations that had been described earlier.

As is evident to the person skilled in the art this new molecular genetic knowledge can now be used to exactly characterize the genotype of the index patient where a given drug takes an unusual effect and of his family.

For the investigation of the nature of the alterations in the amino acid sequence of the CYP3A4, CYP3A7 and hPXR proteins computer programs may be used such as BRASMOL that are obtainable from the Internet. Furthermore, folding simulations and computer redesign of structural motifs can be performed using other appropriate computer programs (Olszewski, *Proteins* 25 (1996), 286-299; Hoffman, *Comput. Appl. Biosci.* 11 (1995), 675-679). Computers can be used for the conformational and energetic analysis of detailed protein models (Monge, *J. Mol. Biol.* 247 (1995), 995-1012; Renouf, *Adv. Exp. Med. Biol.* 376 (1995), 37-45). These analysis can be used for the identification of the influence of a particular mutation on binding and/or metabolism of drugs.

Usually, said amino acid deletion, addition or substitution in the amino acid sequence of the protein encoded by the polynucleotide of the invention is due to one or more nucleotide substitution, insertion or deletion, or any combinations thereof. Preferably said nucleotide substitution, insertion or deletion results in an amino acid substitution of Gly56 to Asp in exon 3 of the CYP3A4 gene and/or Thr409 to Arg in exon 11 of the CYP3A7 gene.

The polynucleotide of the invention may further comprise at least one nucleotide and optionally amino acid deletion, addition and/or substitution other than those specified hereinabove, for example those described in the prior art; e.g.,(13). This embodiment of the present invention allows the study of synergistic effects of the mutations in the CYP3A4, CYP3A7 or hPXR gene on the pharmacological profile of drugs in patients who bear such mutant forms of the gene or similar mutant forms that can be mimicked by the above described proteins. It is expected that the analysis of said synergistic effects provides deeper insights into drug tolerant or sensitive phenotypes of certain forms of cancer and other diseases. From said deeper insight the development of diagnostic and pharmaceutical compositions related to cancer will greatly benefit.

Thus, in a preferred embodiment, the present invention relates to polynucleotides of molecular variant CYP3A4, CYP3A7 and hPXR genes, wherein the nucleotide deletion, addition and/or substitution result in altered expression of the variant CYP3A4, CYP3A7 or hPXR gene compared to the corresponding wild type gene.

Cold Spring Harbor Laboratory (1989) N.Y. and Ausubel, Current Protocols in Molecular Biology, Green Publishing Associates and Wiley Interscience, N.Y. (1994). Alternatively, the polynucleotides and vectors of the invention can be reconstituted into liposomes for delivery to target cells.

The present invention furthermore relates to host cells transformed with a polynucleotide or vector of the invention. Said host cell may be a prokaryotic or eukaryotic cell; see *supra*. The polynucleotide or vector of the invention which is present in the host cell may either be integrated into the genome of the host cell or it may be maintained extrachromosomally. In this respect, it is also to be understood that the recombinant DNA molecule of the invention can be used for "gene targeting" and/or "gene replacement", for restoring a mutant gene or for creating a mutant gene via homologous recombination; see for example Mouellic, Proc. Natl. Acad. Sci. USA, 87 (1990), 4712-4716; Joyner, Gene Targeting, A Practical Approach, Oxford University Press.

The host cell can be any prokaryotic or eukaryotic cell, such as a bacterial, insect, fungal, plant, animal or human cell. Preferred fungal cells are, for example, those of the genus *Saccharomyces*, in particular those of the species *S. cerevisiae*. The term "prokaryotic" is meant to include all bacteria which can be transformed or transfected with a polynucleotide for the expression of a variant CYP3A4, CYP3A7 or hPXR protein or fragment thereof. Prokaryotic hosts may include gram negative as well as gram positive bacteria such as, for example, *E. coli*, *S. typhimurium*, *Serratia marcescens* and *Bacillus subtilis*. A polynucleotide coding for a mutant form of CYP3A4, CYP3A7 and hPXR variant proteins can be used to transform or transfect the host using any of the techniques commonly known to those of ordinary skill in the art. Methods for preparing fused, operably linked genes and expressing them in bacteria or animal cells are well-known in the art (Sambrook, *supra*). The genetic constructs and methods described therein can be utilized for expression of variant CYP3A4, CYP3A7 and hPXR proteins in, e.g., prokaryotic hosts. In general, expression vectors containing promoter sequences which facilitate the efficient transcription of the inserted polynucleotide are used in connection with the host. The expression vector typically contains an origin of replication, a promoter, and a terminator, as well as specific genes which are capable of providing phenotypic selection of the transformed cells. The transformed prokaryotic hosts can be grown in fermentors and cultured according to techniques known in the art to achieve optimal cell growth. The

art. By providing the variant CYP3A4, CYP3A7 and hPXR proteins according to the present invention it is also possible to determine the portions relevant for their biological activity or inhibition of the same.

The present invention furthermore relates to antibodies specifically recognizing a variant CYP3A4, CYP3A7 or hPXR protein according to the invention. Advantageously, the antibody specifically recognizes an epitope containing one or more amino acid substitution(s) as defined above

Antibodies against the variant CYP3A4, CYP3A7 or hPXR protein of the invention can be prepared by well known methods using a purified protein according to the invention or a (synthetic) fragment derived therefrom as an antigen. Monoclonal antibodies can be prepared, for example, by the techniques as originally described in Köhler and Milstein, *Nature* 256 (1975), 495, and Galfré, *Meth. Enzymol.* 73 (1981), 3, which comprise the fusion of mouse myeloma cells to spleen cells derived from immunized mammals. The antibodies can be monoclonal antibodies, polyclonal antibodies or synthetic antibodies as well as fragments of antibodies, such as Fab, Fv or scFv fragments etc. Furthermore, antibodies or fragments thereof to the aforementioned polypeptides can be obtained by using methods which are described, e.g., in Harlow and Lane "Antibodies, A Laboratory Manual", CSH Press, Cold Spring Harbor, 1988. These antibodies can be used, for example, for the immunoprecipitation and immunolocalization of the variant CYP3A4, CYP3A7 and hPXR proteins of the invention as well as for the monitoring of the presence of such variant CYP3A4, CYP3A7 and hPXR proteins, for example, in transgenic organisms, and for the identification of compounds interacting with the proteins according to the invention. For example, surface plasmon resonance as employed in the BIAcore system can be used to increase the efficiency of phage antibodies which bind to an epitope of the protein of the invention (Schier, *Human Antibodies Hybridomas* 7 (1996), 97-105; Malmborg, *J. Immunol. Methods* 183 (1995), 7-13).

Furthermore, the present invention relates to nucleic acid molecules which represent or comprise the complementary strand of any of the above described polynucleotides or a part thereof, thus comprising at least one nucleotide difference compared to the corresponding wild type CYP3A4, CYP3A7 and hPXR gene nucleotide sequences specified by the above described nucleotide substitutions, deletions and additions. Such a

numerous utilities, including as a research model for drug tolerability and therefore, presents a novel and valuable animal in the development of therapies, treatment, etc. for diseases caused by deficiency or failure of drug metabolism in the cell. Accordingly, in this instance, the mammal is preferably a laboratory animal such as a mouse or rat.

Preferably, the transgenic non-human animal of the invention further comprises at least one inactivated wild type allele of the CYP3A4, CYP3A7 and/or hPXR gene. This embodiment allows for example the study of the interaction of various variant forms of CYP3A4, CYP3A7 and hPXR proteins. It might be also desirable to inactivate CYP3A4, CYP3A7 and/or hPXR gene expression or function at a certain stage of development and/or life of the transgenic animal. This can be achieved by using, for example, tissue specific, developmental and/or cell regulated and/or inducible promoters which drive the expression of, e.g., an antisense or ribozyme directed against the RNA transcript of the CYP3A4, CYP3A7 or hPXR gene; see also *supra*. A suitable inducible system is for example tetracycline-regulated gene expression as described, e.g., by Gossen and Bujard (Proc. Natl. Acad. Sci. 89 USA (1992), 5547-5551) and Gossen et al. (Trends Biotech. 12 (1994), 58-62). Similar, the expression of the variant CYP3A4, CYP3A7 and hPXR gene may be controlled by such regulatory elements.

With the variant CYP3A4, CYP3A7 and hPXR polynucleotides and proteins and vectors of the invention, it is now possible to study *in vivo* and *in vitro* the efficiency of drugs in relation to particular mutations in the CYP3A4, CYP3A7 or hPXR gene of a patient and the affected phenotype. Furthermore, the variant CYP3A4, CYP3A7 and hPXR proteins of the invention can be used to determine the pharmacological profile of drugs and for the identification and preparation of further drugs which may be more effective for the treatment of, e.g., cancer, in particular for the amelioration of certain phenotypes caused by the respective mutations such as those described above.

Thus, a particular object of the present invention concerns drug/pro-drug selection and formulation of pharmaceutical compositions for the treatment of diseases which are amenable to chemotherapy taking into account the polymorphism of the variant form of the CYP3A4, CYP3A7 or hPXR gene that cosegregates with the affected phenotype of the patient to be treated. This allows the safe and economic application of drugs which for

It can then be determined whether said sample or compound displays the desired properties, for example, by the methods described herein or in the literature (e.g. (13) and Lehmann, J Clin Invest 102 (1998), 1016-23). Depending on the complexity of the samples, the steps described above can be performed several times, preferably until the sample identified according to the method of the invention only comprises a limited number of or only one substance(s). Preferably said sample comprises substances of similar chemical and/or physical properties, and most preferably said substances are identical. The methods of the present invention can be easily performed and designed by the person skilled in the art, for example in accordance with other cell based assays described in the prior art or by using and modifying the methods as described herein. Furthermore, the person skilled in the art will readily recognize which further compounds and/or enzymes may be used in order to perform the methods of the invention, for example, enzymes, if necessary, that convert a certain compound into the precursor which in turn represents a substrate for the CYP3A4 or CYP3A7 protein. Such adaptation of the method of the invention is well within the skill of the person skilled in the art and can be performed without undue experimentation.

Suitable assays which can be employed in accordance with the present invention are described, for example, in Hashimoto, Eur J Biochem 218 (1993), 585-95 wherein transfection assays with chimeric CYP3A4 genes in HepG2 cells are described. Similarly, the variant CYP3A4, CYP3A7 and/or hPXR genes can be expressed or co-expressed in HepG2 cells and analyzed for their transcriptional activity and catalytic properties of CYP3A4 or CYP3A7. Such an assay can also be used for studying the catalytic properties of the CYP3A4 and CYP3A7 on its substrates such as steroids (testosterone, progesterone, androstenedione, cortisol,  $17\beta$ -oestradiol,  $17\alpha$ -ethynyloestradiol), antibiotics (erythromycin), immunosuppressive (cyclosporine A), benzodiazepine (midazolam), benzothiazepine derivatives (diltiazem, triazolam), and nifedipine. In particular, such tests are useful to add in predicting whether a given drug will interact in an individual carrying the respective variant CYP3A4, CYP3A7 and/or hPXR gene. A suitable expression system which can be employed in accordance with above described methods of the present invention is also described in (22). In addition heterologous expression systems such as yeast can be used in order to study the stability, binding properties and catalytic activities of the gene products of the variant CYP3A4, CYP3A7 and hPXR genes compared to the

(1996), 220-234 and Dorner, Bioorg. Med. Chem. 4 (1996), 709-715. Furthermore, the three-dimensional and/or crystallographic structure of inhibitors and the CYP3A4, CYP3A7 or hPXR protein of the invention can be used for the design of peptidomimetic drugs (Rose, Biochemistry 35 (1996), 12933-12944; Rutenberg, Bioorg. Med. Chem. 4 (1996), 1545-1558).

In summary, the present invention provides methods for identifying and obtaining compounds which can be used in specific doses for the treatment of specific forms of diseases, e.g., cancer the chemotherapy of which is complicated by malfunctions of the CYP3A4, CYP3A7 or hPXR gene often resulting in an altered activity or level of drug metabolism or sensitive phenotype.

In a preferred embodiment of the method of the invention said cell is a cell of or, obtained by the method of the invention or is comprised in the above-described transgenic non-human animal.

In a further embodiment the present invention relates to a method of identifying and obtaining an CYP3A4, CYP3A7 or hPXR inhibitor capable of modulating the activity of a molecular variant of the CYP3A4, CYP3A7 or hPXR gene or its gene product comprising the steps of

- (a) contacting the variant CYP3A4, CYP3A7 or hPXR protein of the invention with a first molecule known to be bound by CYP3A4, CYP3A7 or hPXR protein to form a first complex of said protein and said first molecule;
- (b) contacting said first complex with a compound to be screened; and
- (c) measuring whether said compound displaces said first molecule from said first complex.

Advantageously, in said method said measuring step comprises measuring the formation of a second complex of said protein and said inhibitor candidate. Preferably, said measuring step comprises measuring the amount of said first molecule that is not bound to said protein.



A Laboratory Manual" second ed., CSH Press, Cold Spring Harbor, 1989; "Nucleic Acid Hybridisation, A Practical Approach", Hames and Higgins eds., IRL Press, Oxford, 1985. Furthermore, the mRNA, cRNA, cDNA or genomic DNA obtained from the subject may be sequenced to identify mutations which may be characteristic fingerprints of mutations in the CYP3A4, CYP3A7 and hPXR gene. The present invention further comprises methods wherein such a fingerprint may be generated by RFLPs of DNA or RNA obtained from the subject, optionally the DNA or RNA may be amplified prior to analysis, the methods of which are well known in the art. RNA fingerprints may be performed by, for example, digesting an RNA sample obtained from the subject with a suitable RNA-Enzyme, for example RNase T<sub>1</sub>, RNase T<sub>2</sub> or the like or a ribozyme and, for example, electrophoretically separating and detecting the RNA fragments as described above. Further modifications of the above-mentioned embodiment of the invention can be easily devised by the person skilled in the art, without any undue experimentation from this disclosure; see, e.g., the examples. An additional embodiment of the present invention relates to a method wherein said determination is effected by employing an antibody of the invention or fragment thereof. The antibody used in the method of the invention may be labeled with detectable tags such as a histidine flags or a biotin molecule.

In a preferred embodiment of the present invention, the above described methods comprise PCR, ligase chain reaction, restriction digestion, direct sequencing, nucleic acid amplification techniques, hybridization techniques or immunoassays (Sambrook et al., loc. cit. CSH cloning, Harlow and Lane loc. cit. CSH antibodies).

In a preferred embodiment of the method of the present invention said disorder is cancer.

In a further embodiment of the above-described method, a further step comprising administering to the subject a medicament to abolish or alleviate said variations in the CYP3A4, CYP3A7 or hPXR gene in accordance with all applications of the method of the invention allows treatment of a given disease before the onset of clinical symptoms due to the phenotype response caused by the CYP3A4, CYP3A7 or hPXR gene.

In a preferred embodiment of the method of the invention said medicament are chemotherapeutic agents such as substrates of CYP3A4: paclitaxen (Eur J Drug Metab



and administered to a patient as discussed above. For uses and therapeutic doses determined to be appropriate by one skilled in the art see *infra*.

Furthermore, the present invention relates to a method for the preparation of a pharmaceutical composition comprising the steps of the above-described methods; and formulating a drug or pro-drug in the form suitable for therapeutic application and preventing or ameliorating the disorder of the subject diagnosed in the method of the invention.

Drugs or pro-drugs after their *in vivo* administration are metabolized in order to be eliminated either by excretion or by metabolism to one or more active or inactive metabolites (Meyer, J. Pharmacokinet. Biopharm. 24 (1996), 449-459). Thus, rather than using the actual compound or inhibitor identified and obtained in accordance with the methods of the present invention a corresponding formulation as a pro-drug can be used which is converted into its active in the patient. Precautionary measures that may be taken for the application of pro-drugs and drugs are described in the literature; see, for review, Ozama, J. Toxicol. Sci. 21 (1996), 323-329).

In a preferred embodiment of the method of the present invention said drug or prodrug is a derivative of a medicament as defined hereinbefore.

In a still further embodiment the present invention relates to an inhibitor identified or obtained by the method described hereinbefore. Preferably, the inhibitor binds specifically to the variant CYP3A4, CYP3A7 or hPXR protein of the invention. The antibodies, nucleic acid molecules and inhibitors of the present invention preferably have a specificity at least substantially identical to binding specificity of the natural ligand or binding partner of the CYP3A4, CYP3A7 or hPXR protein of the invention. An antibody or inhibitor can have a binding affinity to the CYP3A4, CYP3A7 or hPXR protein of the invention of at least  $10^5 \text{ M}^{-1}$ , preferably higher than  $10^7 \text{ M}^{-1}$  and advantageously up to  $10^{10} \text{ M}^{-1}$  in case CYP3A4, CYP3A7 or hPXR activity should be repressed. Hence, in a preferred embodiment, a suppressive antibody or inhibitor of the invention has an affinity of at least about  $10^{-7} \text{ M}$ , preferably at least about  $10^{-9} \text{ M}$  and most preferably at last about  $10^{-11} \text{ M}$ .

detection of the variant CYP3A4, CYP3A7 or hPXR protein of the invention, the expression of a molecular variant CYP3A4, CYP3A7 or hPXR gene comprising a polynucleotide of the invention and/or for distinguishing CYP3A4, CYP3A7 and hPXR alleles comprising a polynucleotide of the invention.

Moreover, the present invention relates to a composition, preferably pharmaceutical composition comprising the antibody, the nucleic acid molecule, the vector or the inhibitor of the present invention, and optionally a pharmaceutically acceptable carrier. These pharmaceutical compositions comprising, e.g., the inhibitor or pharmaceutically acceptable salts thereof may conveniently be administered by any of the routes conventionally used for drug administration, for instance, orally, topically, parenterally or by inhalation. Acceptable salts comprise acetate, methylester, HCl, sulfate, chloride and the like. The compounds may be administered in conventional dosage forms prepared by combining the drugs with standard pharmaceutical carriers according to conventional procedures. These procedures may involve mixing, granulating and compressing or dissolving the ingredients as appropriate to the desired preparation. It will be appreciated that the form and character of the pharmaceutically acceptable character or diluent is dictated by the amount of active ingredient with which it is to be combined, the route of administration and other well-known variables. The carrier(s) must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient thereof. The pharmaceutical carrier employed may be, for example, either a solid or liquid. Exemplary of solid carriers are lactose, terra alba, sucrose, talc, gelatin, agar, pectin, acacia, magnesium stearate, stearic acid and the like. Exemplary of liquid carriers are phosphate buffered saline solution, syrup, oil such as peanut oil and olive oil, water, emulsions, various types of wetting agents, sterile solutions and the like. Similarly, the carrier or diluent may include time delay material well known to the art, such as glyceryl monostearate or glyceryl distearate alone or with a wax.

The dosage regimen will be determined by the attending physician and other clinical factors; preferably in accordance with any one of the above described methods. As is well known in the medical arts, dosages for any one patient depends upon many factors, including the patient's size, body surface area, age, the particular compound to be administered, sex, time and route of administration, general health, and other drugs being administered concurrently. Progress can be monitored by periodic assessment.

them far less capable of facilitating drug metabolization and transcription initiation, respectively. Restoring the normal or regulated conformation of mutated proteins is the most elegant and specific means to correct these molecular defects, although it is difficult. Pharmacological manipulations thus may aim at restoration of wild-type conformation of the protein. Thus, the polynucleotides and encoded proteins of the present invention may also be used to design and/or identify molecules which are capable of activating the wild-type function of a CYP3A4, CYP3A7 or hPXR gene or protein.

In another embodiment the present invention relates to the use of a drug or prodrug for the preparation of a pharmaceutical composition for the treatment or prevention of a disorder diagnosed by the method described hereinbefore.

Furthermore, the present invention relates to the use of an effective dose of a nucleic acid sequence encoding a functional and expressible wild type CYP3A4, CYP3A7 or hPXR protein for the preparation of a pharmaceutical composition for treating, preventing and/or delaying a disorder diagnosed by the method of the invention. A gene encoding a functional and expressible CYP3A4, CYP3A7 or hPXR protein can be introduced into the cells which in turn produce the protein of interest. Gene therapy, which is based on introducing therapeutic genes into cells by *ex-vivo* or *in-vivo* techniques is one of the most important applications of gene transfer. Suitable vectors and methods for *in-vitro* or *in-vivo* gene therapy are described in the literature and are known to the person skilled in the art; see, e.g., Giordano, Nature Medicine 2 (1996), 534-539; Schaper, Circ. Res. 79 (1996), 911-919; Anderson, Science 256 (1992), 808-813; Isner, Lancet 348 (1996), 370-374; Muhlhauser, Circ. Res. 77 (1995), 1077-1086; Wang, Nature Medicine 2 (1996), 714-716; WO94/29469; WO 97/00957 or Schaper, Current Opinion in Biotechnology 7 (1996), 635-640, and references cited therein. The gene may be designed for direct introduction or for introduction via liposomes, or viral vectors (e.g. adenoviral, retroviral) into the cell. Preferably, said cell is a germ line cell, embryonic cell, or egg cell or derived therefrom, most preferably said cell is a stem cell.

As is evident from the above, it is preferred that in the use of the invention the nucleic acid sequence is operatively linked to regulatory elements allowing for the expression and/or targeting of the CYP3A4, CYP3A7 or hPXR protein to specific cells. Suitable gene delivery systems that can be employed in accordance with the invention may include liposomes,

**Brief description of the figures**

- Figure 1:** Differences in the genetic makeup influence the efficacy and safety of drug treatment.
- Figure 2:** A current model of the regulation of CYP3A4 by hPXR.
- Figure 3:** (A) Structure of the CYP3A4 gene as described in Ref. by Hashimoto, Eur Biochem 218 (1993) 585-95 and confirmed in this study. Coding regions are indicated as filled rectangles, the non-coding 5' untranslated region as a dashed rectangle. Arrowheads represent the positions of oligonucleotides used to screen the coding region of the gene (see Table 2 for the oligonucleotide sequences). (B) Determination of the exon flanking sequences. Double-headed arrows indicate genomic regions amplified by PCR. Single-headed arrows indicate sequences obtained by direct sequencing of BAC clones. The sequences of oligonucleotides shown in (B) are given in Table 1.
- Figure 4:** (A) Structure of the hPXR gene. Coding regions are indicated as filled rectangles, non-coding 5' and 3' untranslated regions as dashed rectangles. Arrowheads represent the positions of oligonucleotides used to screen the coding region of the gene (see Table 5 for the oligonucleotide sequences). (B) Elucidation of the genomic structure of hPXR. Double-headed arrows indicate genomic regions amplified by PCR. Oligonucleotides used to isolate an hPXR-containing BAC clone are shown in bold. Single-headed arrows indicate sequences obtained by direct sequencing of this clone. (C) Differential expression of hPXR transcripts in the liver (L) and small intestine (SI) as investigated by PCR amplification of tissue-derived cDNA pools. Exon 1b is expressed in both target tissues of the gene whereas exon 1a is expressed only in the liver. The sequences of oligonucleotides shown in (B) and (C) are given in Table 3.

obtained under consideration of all legal, ethical and medical requirement of Parexel International (Berlin).

**Example 1: Genomic organization and oligonucleotides for the amplification of the coding regions of CYP3A4, CYP3A7 and hPXR**

The genomic structure of CYP3A4 has been described in an earlier work (Hashimoto, Eur. J. Biochem. 218 (1993), 585-95); however, the published exon flanking sequences were too short to design oligonucleotides for exon amplification. In accordance with the present invention these sequences have been elucidated first by sequencing of PCR (Polymerase Chain Reaction)-amplified fragments containing parts of two neighboring exons and the intercalated intron. The primers used for the amplification of these fragments were derived from the published cDNA sequence (Beaune, Proc. Natl. Acad. Sci. USA 83 (1986), 8064-8, GenBank accession number M14096) upon consideration of the exon/intron organization of the gene (Table 1, Fig. 3). For exons flanked by larger introns, CYP3A4-containing bacterial artificial chromosome (BAC) clones (Control numbers 22300 and 22301) purchased from Genome Systems (St. Louis, MO, USA) were directly sequenced. The clones had been isolated by PCR using the CYP3A4PF and CYP3A4PR oligonucleotides (Table 1), which were derived from the promoter region of the gene (GenBank accession number D11131, Hashimoto, supra). The sequences thus obtained were used to design oligonucleotides for the amplification of the gene exons (Fig. 1). Their sequences, the amplification conditions, and the sizes of the resulting DNA fragments are given in Table 2. Besides the exon sequences, fragments amplified contain also some flanking intronic sequences, including the splice sites. The oligonucleotide 3A425R was derived from the non-coding part of CYP3A4 cDNA (exon 13, Beaune, supra, GenBank accession number M14096). The sequences of oligonucleotides to determine the exon/intron boundary of Exon 11 of CYP3A7 (3A720R and 3A721F) are shown in **bold** in Table 1.

In accordance with the present invention a similar strategy was applied to elucidate the genomic organization and to determine exon-flanking sequences of the CYP3A4 and CYP3A7 regulator, hPXR (Fig. 4A, B, Table 3). The gene consists of 10 exons and spans at least 19 kb of genomic DNA. The sequences at exon-intron junctions as well as exon and intron sizes are given in Table 4. Exons 1b and 1a are utilized alternately in the

### Example 3: Polymorphisms in the CYP3A4 and CYP3A7 gene

In accordance with the present invention, 22 or more DNA samples were screened for mutations in exons 3, 4, 5, 7 and 9 of the CYP3A4 gene, and in exon 11 of the CYP3A7 gene. Three novel mutations were detected in the samples screened, two of them result in non-conservative amino acid exchanges (Fig. 5, Table 6, Table 8, Figure 8). The numbering of nucleotide polymorphisms within the CYP3A4 transcript, and of positions of the resulting amino acid exchanges, is based on GenBank sequence M14096. For CYP3A7 GenBank sequence gi 4503232 was used as reference.

- a G235A single nucleotide polymorphism (SNP) in exon 3 of CYP3A4 in 6 of the 234 chromosomes screened. The SNP results in the non-conservative amino acid exchange Gly→Asp at position 56 of the CYP3A4 protein.
- a G→T SNP in intron 7 of CYP3A4 in 4 out of 44 chromosomes screened. The SNP is localized at position 33 of the intron sequence (the G of the 5' consensus splice site GT is taken as position 1).
- a C1229G SNP in exon 11 of CYP3A7 in 17 out of 232 chromosomes screened. The SNP results in the non-conservative amino acid exchange Thr→Arg at position 409 of the CYP3A7 protein.

### Example 4: Polymorphisms in the hPXR gene

In accordance with the present invention 22 DNA samples were screened for mutations in exons 1b, 2, 5/6, 7, 8, 9 and 9a of the hPXR gene. Three polymorphisms were detected in the samples screened (Fig. 6, Table 7, Table 8, Figure 8). The numbering of nucleotide polymorphisms within the hPXR transcript, and of positions of the resulting amino acid exchanges is based on GenBank hPXR sequence AF084645.

- A C149A SNP in exon 1b in 27 out of 44 chromosomes screened. Exon 1b is untranslated, i.e. the SNP has no effect on the amino acid sequence of the protein.
- a CC975TT SNP in exon 5/6 in 2 out of 44 chromosomes screened. The SNP is silent, i.e. it has no effect on the amino acid sequence of the protein.
- A C→T SNP in intron 7 in 8 out of 44 chromosomes screened. The SNP is localized at position 184 of the intron sequence (the G of the 5' consensus splice site GT is taken as position 1).

|          |           |                          |
|----------|-----------|--------------------------|
| 3A416R   | exon 9    | CCTTTGTGGGACTCAGTTTC     |
| 3A419F   | exon 10   | GCCACTCACCCTGATGTC       |
| 3A720R   | exon 11   | ATCACCACCCACCCTTTG       |
| 3A721F   | exon 11   | CAAAGGGTGGGTGGTGAT       |
| 3A422R   | exon 12   | GAGAGCAAACCTCATGCC       |
| 3A423F   | exon 12   | GGCATGAGGTTTGCTCTC       |
| 3A424R   | exon 13   | GGTGCCATCCCTTGA CT C     |
| 3A426R   | exon 2    | GCAGAGGTGTGGGCCCTG       |
| 3A4436F  | intron 8  | GGAGATCAAGGACCACGCTTG TG |
| 3A441R   | intron 10 | CTTACGCTTCTGCCAGTAGCAACC |
| CYP3A4PF | promoter  | AACAGGCGTGGAACACAAT      |
| CYP3A4PR | promoter  | CTTTCCTGCCCTGCACAG       |

Fifty ng of genomic DNA was added to a reaction mix (total volume 30 or 50 µl) containing 1x PCR buffer (Q=Qiagen, Cat.Nr. 1005479, or B2=Boehringer (currently Roche) Expand Long Template PCR Buffer number 2, Cat. Nr. 1742655), 0.25 µM each oligonucleotide (Metabion), 200 µM dNTPs, and 1 U of Taq polymerase (Qiagen). Amplifications were performed on a RoboCycler Gradient 96 (Stratagene) with an initial denaturation step of 2 min. at 94 °C followed by 32 amplification cycles of denaturation (40 sec., 94 °C), annealing (45 sec., temperatures 56-60 °C), and extension (60-150 sec., 72 °C). This was followed by a final extension step 5 min., 72 °C. The sequencing of PCR fragments and BAC clones was performed on a GeneAmp PCR System 9700 (Perkin-Elmer) using a dye-terminator DNA sequencing kit. (Perkin-Elmer, Cat.Nr. 4303154), according to manufacturers's instructions.

Fifty ng of genomic DNA was added to a reaction mix containing 1x PCR buffer (Qiagen), 0.5  $\mu$ M oligonucleotides, 200  $\mu$ M dNTPs, and 1 U of Taq polymerase (Qiagen). Amplifications were performed on a RoboCycler Gradient 96 (Stratagene) with an initial denaturation step of 2 min. at 94 °C followed by 32 amplification cycles of denaturation (40 sec., 94 °C), annealing (45 sec., temperatures given above), and extension (60 sec., 72 °C). This was followed by a final extension step 5 min., 72 °C. All sequencing reactions were performed on a GeneAmp PCR System 9700 (Perkin-Elmer) using a dye-terminator DNA sequencing kit (Perkin-Elmer, catalog number 4303154), according to manufacturers's instructions.

**Table 3.** Oligonucleotides used to determine the structure and exon/intron boundaries of the hPXR gene.

| Name    | Position | Sequence (5' - 3')       |
|---------|----------|--------------------------|
| hPXR1F  | exon4    | TCATGTCCGACGAGGCCG       |
| hPXR4F  | exon 5/6 | CCCACATGGCTGACATGT       |
| hPXR5F  | exon 7   | CCCATCGAGGACCAGATC       |
| hPXR6R  | exon 7   | GTCTTCCAAGCAGTAGGA       |
| hPXR7R  | exon 8   | CAGCATGGGCTCCAGTAG       |
| hPXR10R | exon 9a  | CCTGTGATGCCGAACAAC       |
| hPXR11F | exon 9   | CATTGAATGCAATCGGCC       |
| hPXR12R | exon 9a  | GCTCTTGGCAGTGTCCAT       |
| hPXR15F | exon 2   | GGAAAGCCCAGTGTCAAC       |
| hPXR16F | exon 3   | CCATGAAACGCAACGCCC       |
| hPXR18R | exon 2   | CCTTGCACTCCTTCACATG      |
| hPXR19R | exon 3   | CATGCCGCTCTCCAGGCA       |
| hPXR20R | exon 4   | CGGCCTCGTCGGACATGA       |
| hPXR21R | exon 5/6 | ACATGTCAGCCATGTGGG       |
| hPXR47F | exon 1b  | CAAGCCAAGTGTTACAGTG      |
| hPXR48R | exon 1b  | CACTGTGAACACTTGGCTTG     |
| hPXR52F | exon 1a  | CAAGGACAGCAGCATGACAGTCAC |
| hPXR54R | exon 1a  | AGCCAACTCAGCCGCAGC       |



Table 5. hPXR polymorphism screen: oligonucleotide sequences, amplification conditions and fragment size.

| Exon | Upstr am Oligonucleotide |                      | Downstream Oligonucleotide |                       | Ann. Temp. (°C) | Buffer | Product Size (bp) |
|------|--------------------------|----------------------|----------------------------|-----------------------|-----------------|--------|-------------------|
|      | Name                     | Sequence (5'-3')     | Name                       | Sequence (5'-3')      |                 |        |                   |
| 1b   | HPXR57F                  | TCAAGTGCTGGACTTGGGAC | HPXR58R                    | CCCACATATGATGCTGACCTC | 53              | B2     | 460               |
| 1a   |                          |                      |                            |                       |                 |        |                   |
| 2    | HPXR41F                  | CTGAGGCCTCTACACATC   | HPXR40R                    | AGGCCCTGAGATGTTACC    | 55              | Q      | 345               |
| 3    |                          |                      |                            |                       |                 |        |                   |
| 4    |                          |                      |                            |                       |                 |        |                   |
| 5/6  | HPXR32F                  | CTGAGTTGGGACCTGTCT   | HPXR35R                    | CCAGGCCCTTTGAACCTC    | 60              | B2     | 415               |
| 7&8  | HPXR36F                  | CTGCTGGTGCCCGCCTGT   | HPXR33R                    | GACTGGGACCTTCCCTGG    | 60              | B2     | 598               |
| 9    | HPXR34F                  | GAGCAATGCCCTGACTCT   | HPXR26R                    | CCCTCTGGCCCATGAAATC   | 60              | B2     | 271               |
| 9a   | HPXR30F                  | TGCTTGTCAGCCTCAGA    | HPXR12R                    | GCTCTTGGCAGTGTCAT     | 60              | B2     | 324               |

Preliminary exon numbering. Fifty ng of genomic DNA was added to a reaction mix (total volume 30 or 50 µl) containing 1x PCR buffer (Q=Qiagen, Cat.Nr. 1005479, or B2=Boehringer (currently Roche) Expand Long Template PCR Buffer number 2, Cat. Nr. 1742655), 0.25 µM each oligonucleotide (Metabion), 200 µM dNTPs, and 1 U of Taq polymerase (Qiagen). Amplifications were performed on a RoboCycler Gradient 96 (Stratagene) with an initial denaturation step of 2 min. at 94 °C followed by 32 amplification cycles of denaturation (40 sec., 94 °C), annealing (45 sec., temperatures given above), and extension (60 sec., 72 °C). This was followed by a final extension step 5 min., 72 °C All sequencing reactions were performed on a GeneAmp PCR System 9700 (Perkin-Elmer) using a dye-terminator DNA sequencing kit (Perkin-Elmer, Cat.Nr. 4303154), according to manufacturers's instructions.

**Tabl 7.** Distribution of hPXR gene polymorphisms in a set of 22 DNA samples.

| Number | Donor  | Exons |    |   |   |   |     |    |   |   |    |
|--------|--------|-------|----|---|---|---|-----|----|---|---|----|
|        | Number | 1b    | 1a | 2 | 3 | 4 | 5/6 | 7  | 8 | 9 | 9a |
| 1      | 11     |       |    | - |   |   | -   | X  | - | - | -  |
| 2      | 17     | X     |    | - |   |   | -   | -  | - | - | -  |
| 3      | 18     | XX    |    | - |   |   | -   | -  | - | - | -  |
| 4      | 19     | X     |    | - |   |   | -   | -  | - | - | -  |
| 5      | 20     | X     |    | - |   |   | -   | -  | - | - | -  |
| 6      | 21     | XX    |    | - |   |   | -   | X  | - | - | -  |
| 7      | 22     | XX    |    | - |   |   | -   | -  | - | - | -  |
| 8      | 23     | XX    |    | - |   |   | -   | -  | - | - | -  |
| 9      | 24     | XX    |    | - |   |   | -   | -  | - | - | -  |
| 10     | 25     | X     |    | - |   |   | -   | -  | - | - | -  |
| 11     | 27     | -     |    | - |   |   | XX  | XX | - | - | -  |
| 12     | 29     | XX    |    | - |   |   | -   | X  | - | - | -  |
| 13     | 31     | X     |    | - |   |   | -   | X  | - | - | -  |
| 14     | 33     | -     |    | - |   |   | -   | -  | - | - | -  |
| 15     | 34     | X     |    | - |   |   | -   | -  | - | - | -  |
| 16     | 35     | XX    |    | - |   |   | -   | -  | - | - | -  |
| 17     | 36     | X     |    | - |   |   | -   | -  | - | - | -  |
| 18     | 37     | X     |    | - |   |   | -   | -  | - | - | -  |
| 19     | 38     | -     |    | - |   |   | -   | X  | - | - | -  |
| 20     | 40     | XX    |    | - |   |   | -   | X  | - | - | -  |
| 21     | 41     | X     |    | - |   |   | -   | -  | - | - | -  |
| 22     | 43     | XX    |    | - |   |   | -   | -  | - | - | -  |

"-"=wild-type, "X"=heterozygote, "XX"=homozygote

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**Claims**

1. A polynucleotide selected from the group consisting of:
  - (a) polynucleotides comprising a nucleotide sequence encoding the amino acid sequence encodable by a nucleotide sequence of SEQ ID NO: 86, 94 and/or 98;
  - (b) polynucleotides comprising a nucleotide sequence encoding a molecular variant of the cytochrome CYP3A4 protein by way of at least one amino acid deletion, addition and/or substitution at an amino acid position corresponding amino acid residue Gly56 in exon 3 of the CYP3A4 gene;
  - (c) polynucleotides comprising a nucleotide sequence encoding a molecular variant of the cytochrome CYP3A7 protein by way of at least one amino acid deletion, addition and/or substitution, preferably at an amino acid position corresponding amino acid residue Thr 409 in exon 11 of the CYP3A7 gene;
  - (d) polynucleotides comprising a nucleotide sequence of any one of SEQ ID NOS: 86, 87, 90, 91, 94, 95 or 108;
  - (e) polynucleotides comprising a nucleotide sequence of any one of SEQ ID NOS: 98, 99, 102, 103, 106 or 107; and
  - (f) polynucleotides comprising a nucleotide sequence encoding a CYP3A4 or CYP3A7 polypeptide or fragment thereof having an epitope comprising an amino acid sequence encoded by the nucleotide sequence of SEQ ID NO: 86 and/or 94.
2. The polynucleotide of claim 1, wherein said polynucleotide encodes a variant CYP3A4, CYP3A7 or hPXR protein or fragment thereof.
3. The polynucleotide of claim 1 or 2, wherein said amino acid substitution comprises Gly56 to Asp in exon 3 of the CYP3A4 gene and/or Thr409 to Arg in exon 11 of the CYP3A7 gene.
4. The polynucleotide of any one of claims 1 to 3, wherein the nucleotide deletion, addition and/or substitution result in altered expression of the variant CYP3A4, CYP3A7 or hPXR gene compared to the corresponding wild type gene.

16. A transgenic non-human animal comprising at least one polynucleotide of any one of claims 1 to 4 or the vector of claim 5 or 6.
17. The transgenic non-human animal of claim 16 further comprising at least one inactivated wild type allele of the CYP3A4, CYP3A7 or hPXR gene.
18. The transgenic non-human animal of claim 16 or 17, which is a mouse or a rat.
19. A method of identifying and obtaining a CYP3A4, CYP3A7 or hPXR inhibitor capable of modulating the activity of a molecular variant of the CYP3A4, CYP3A7 or hPXR gene or its gene product comprising the steps of
  - (a) contacting the protein of claim 10 or a cell expressing a molecular variant CYP3A4, CYP3A7 or hPXR gene comprising a polynucleotide of any one of claims 1 to 4 in the presence of components capable of providing a detectable signal in response to drug metabolism, with a compound to be screened under conditions to permit CYP3A4- or CYP3A7-mediated drug metabolism, and
  - (b) detecting the presence or absence of a signal or increase of a signal generated from the drug metabolism, wherein the presence or increase of the signal is indicative for a putative inhibitor.
20. The method of claim 19 wherein said cell is a cell of claim 7, obtained by the method of claim 9 or is comprised in the transgenic non-human animal of any one of claims 16 to 18:
21. A method of identifying and obtaining an CYP3A4, CYP3A7 or hPXR inhibitor capable of modulating the activity of a molecular variant of the CYP3A4, CYP3A7 or hPXR gene or its gene product comprising the steps of
  - (a) contacting the protein of claim 10 with a first molecule known to be bound by CYP3A4, CYP3A7 or hPXR protein to form a first complex of said protein and said first molecule;
  - (b) contacting said first complex with a compound to be screened; and
  - (c) measuring whether said compound displaces said first molecule from said first complex.

31. A method for the production of a pharmaceutical composition comprising the steps of the method of any one of claims 19 to 25; and  
(c) synthesizing and/or formulating the compound identified and obtained in step (b) or a derivative thereof in a pharmaceutically acceptable form.
32. A method for the preparation of a pharmaceutical composition comprising formulating a drug or pro-drug in the form suitable for therapeutic application and preventing or ameliorating the disorder of the subject diagnosed in the method of claim 26 or 27.
33. The method of claim 31 or 32 wherein said compound drug or prodrug is a derivative of a medicament as defined in claim 29.
34. An inhibitor identified or obtainable by the method of any one of claims 19 to 25.
35. The inhibitor of claim 34 which binds specifically to the protein of claim 10.
36. Use of an oligo- or polynucleotide for the detection of a polynucleotide of any one of claims 1 to 4 and/or for genotyping of individual CYP3A4, CYP3A7 or hPXR alleles.
37. The use of claim 36 wherein said polynucleotide is a polynucleotide of any one of claims 1 to 4 or a nucleic acid molecule of claim 13 or 14.
38. The use of claim 36 wherein said oligonucleotide is about 15 to 50 nucleotides in length and comprises the nucleotide sequence of any one of SEQ ID NOS: 1 to 107 or a complementary sequence.
39. A primer or probe consisting of an oligonucleotide as defined in claim 38.
40. Use of an antibody or a substance capable of binding specifically to the gene product of an CYP3A4, CYP3A7 or hPXR gene for the detection of the protein of claim 10, the expression of a molecular variant CYP3A4, CYP3A7 or hPXR gene comprising a polynucleotide of any one of claims 1 to 4 and/or for

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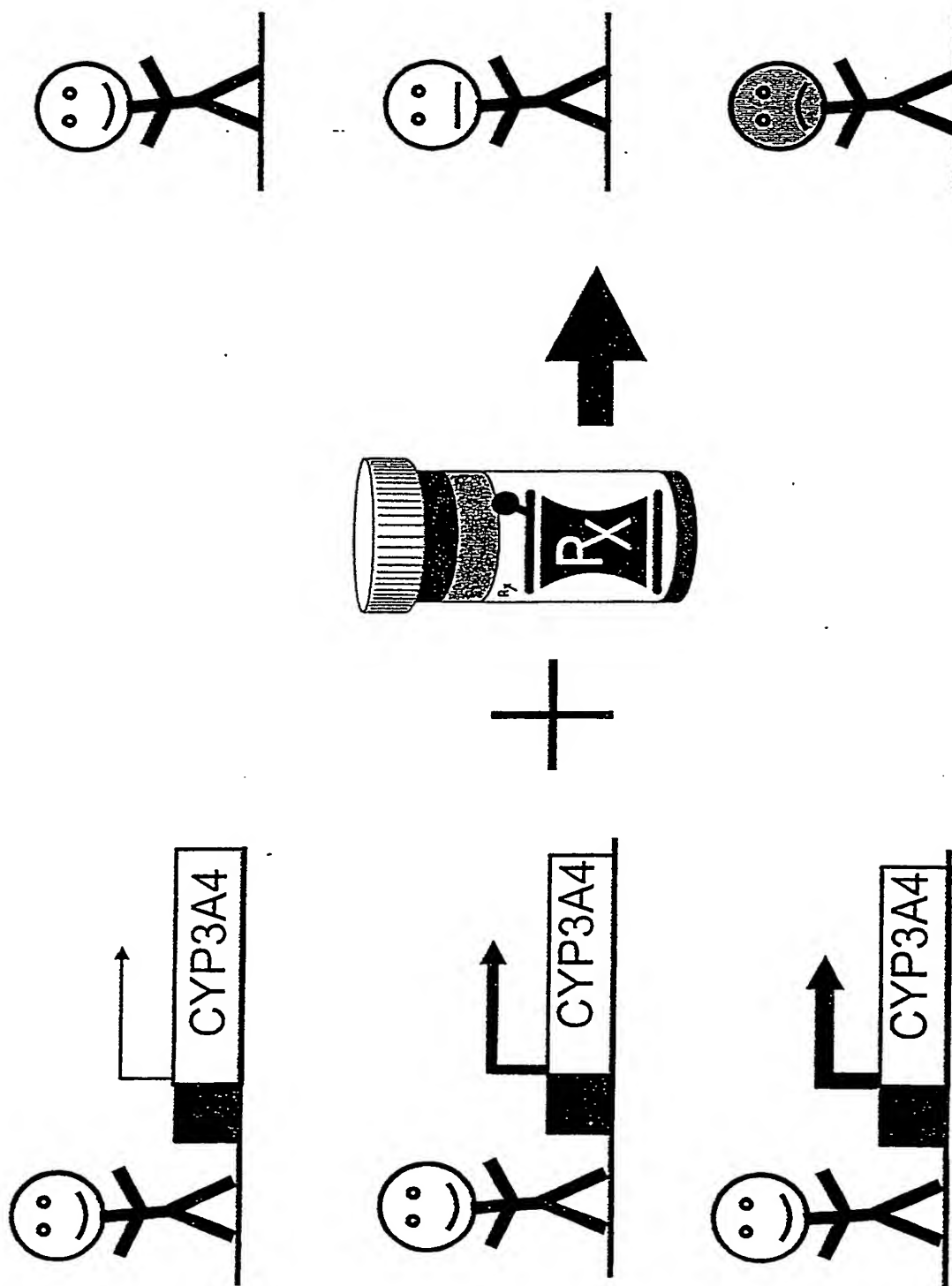


Fig. 1

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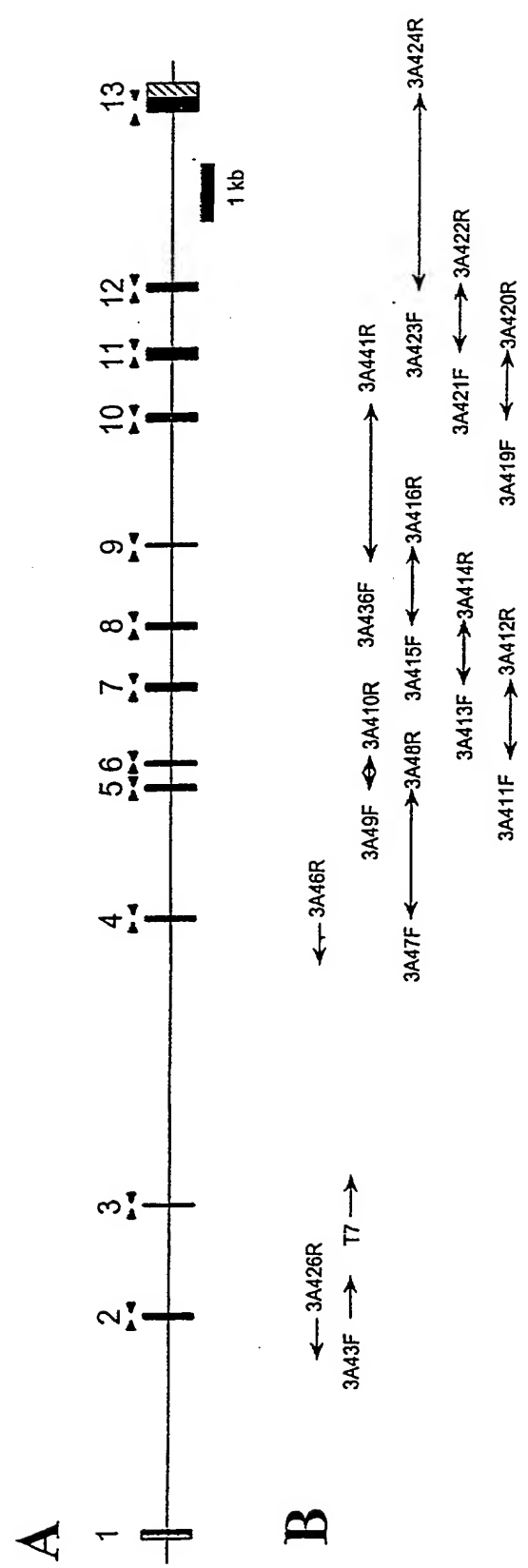


Fig. 3

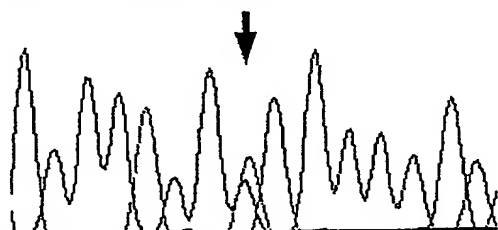


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## CYP3A4 polymorphisms

Exon 3 (G235A; Gly56Asp)

T C C C A G G N C T T T T G T

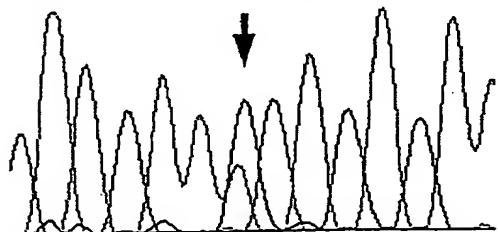


wt/mut

Oligonucleotide 3A450F (forwards)

Intron 7 (T→G)

T A T C T T G C T C T C T T

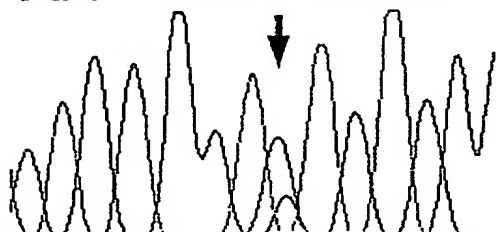


wt/mut

Oligonucleotide 3A433F (forwards)

A CYP3A7 exon 11 polymorphism (C1229G; Thr409Arg)

T A C T G G A C A G A G C C



wt/mut

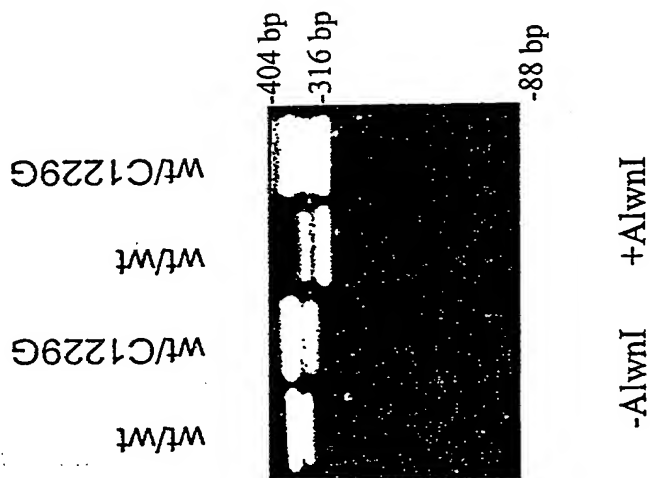
Oligonucleotide 3A742F (forwards)

Fig. 5

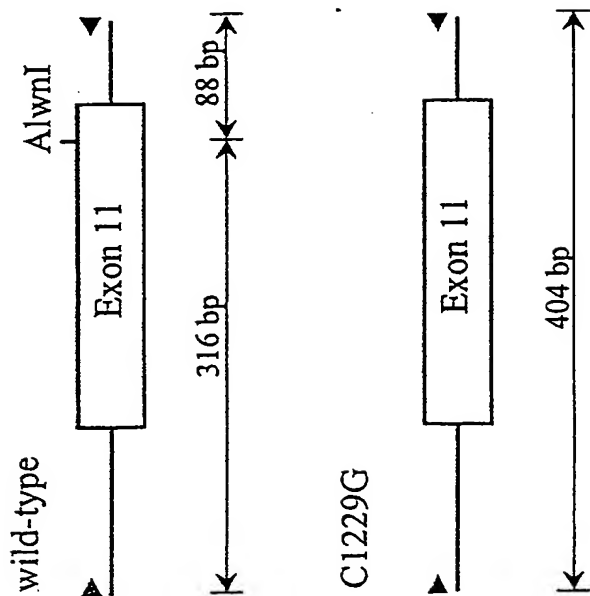
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Fig. 7

B



A



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## Fig. 8 (continued)

## Exon 9

GGAGATCAAGGACCACGCTTGTGATTACTTCTGACTTCAGGAGCCACTTTCTGTCAGT  
GAAATTTCTCTTTTCTTCTAGCACCGAGTGGATTTCTTCAGCTGATGATTGACTCTC  
AGAATTCAAAAGAACTGAGTCCCACAAAGGTAACCAGAGTGTTTCTGAGGGCTACTT  
GTGGGGCACTCAGAGGGAAGGCCTTGTCTGAAAATGTGCAGGAAGTATTCCAGGATG  
ATGAG

CYP3A7

Exon 11 (C1229G Thr409Arg polymorphism)

CCAGTATGAGTTGTTCTCTGGAACCTCTAACAGTTCAACAGTACTACATGGACTGAGTTA  
AAAGTTAATTCAAAAATCTCAATTTATCCAAATCTGTTTCTTTCTTTTCAGGCACCACCCA  
CCTATGATACTGTGCTACAGTTGGAGTATCTTGACATGGTGGTGAATGAAACACTCAG  
ATTATTCCCAGTTGCTATGAGACTTGAGAGGGTCTGCAAAAAAGATGTTGAAATCAAT  
GGGATGTTTATTCCCAAAGGGGTGGTGGTGAATGATTCCAAGCTATGTTCTTCATCATG  
ACCCAAAGTACTGGAC→GAGAGCCTGAGAAGTTCCTCCCTGAAAGGTAGGAGGCCC  
CTGGGAAGGGAGCCCTCCCTGAACCAGCCTGGTTCAAGCATATTCTGCCT

hPXR

Exon 1b&1a (in bold in exon 1b: nn 1-280 of hPXR cDNA (GenBank AF061056.1),  
dotted: nn 36-257 of PAR1 cDNA (GenBank AF084645))

TCAAGTGCTGGACTTGGGACTTAGGAGGGGCAATGGAGCCGCTTAGTGCCTACATCTG  
ACTTGGACTGAAATATAGGTGAGAGACAAGATTGTCTCATATCCGGGGAAATCATAAC  
CTATGACTAGGACGGGAAGAGGAAGCACTGCCTTTACTTCAGTGGGAATCTCGGCCT  
CAGCCTGCAAGCCAAGTGTTACAGTGAAAAAGCAAGAGAATAAGCTAATACTCCT  
GTCCTGAAC→AAAGGCAGCGGCTCCTTGGTAAAGCTACTCCTTGATCGATCCTTTGCA  
CCGGATTGTTCAAAGTGGACCCAGGGGAGAAGTCGGAGCAAAGAACTTACCACCAA  
GCAGGATGGTTTTTCTTTCTTTCTTTTCTGCTGGGGGCTGACCGCCCTTCAGCTCCAG  
CCAAAAGATGTGTGTGAACACAAATATACCTTCTGTT

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Fig. 8 (continued)

GTCCGACGAGGCCGTGGAGGAGAGGCGGGCCTTGATCAAGCGGAAGAAAAGTGAAC  
GGACAGGGACTCAGCCACTGGGAGTGCAGGGGCTGACAGAGGAGCAGCGGATGATG  
ATCAGGGAGCTGATGGACGCTCAGATGAAAACCTTTGACACTACCTTCTCCCATTTC  
AGAATTTCCGGGTAGGAGGAAGTGCACAGTGACCCGAGGTGTCACTGCCATCTTCATT  
CTCACATAGAAACTGAGGTTCCCCAAGGA

## Exon5/6

CTGAGTTGGGACCTGTCTATGAAAGCACATGCTGTCTCTCCTCTGTCCACCTCCTGGCA  
TGTGTCCTAGCTGCCAGGGGTGCTTAGCAGTGGCTGCGAGTTGCCAGAGTCTCTGCA  
GGCCCCATCGAGGGAAGAAGCTGCCAAGTGGAGCCAGGTCCGGAAAGATCTGTGCT  
CTTTGAAGGTCTCTCTGCAGCTGCGGGGGGAGGATGGCAGTGTCTGGAAC TACAAAC  
CCCCAGCCGACAGTGGC→TGGGAAAGAGATCTTCTCCCTGCTGCCCCACATGGCTGA  
CATGTCAACCTACATGTTCAAAGGCATCATCAGCTTTGCCAAAGTCATCTCCTACTTCA  
GGTAGGACATGGAGACTGGGTGTTGGGTGTGGAAAGAACTGGAAGTGGCCAGGAG  
GTTCAAAGGGCCTGG

## EXONS 7&amp;8

CTGCTGGTGCCGGCCTGTGGGCTGCCTCCCAGGGAGCTGTCCTCCCCTCCCCATCCTT  
GCTGCCAGGGACTTGCCCATCGAGGACCAGATCTCCCTGCTGAAGGGGGCCGCTTTC  
GAGCTGTGTCAACTGAGATTCAACACAGTGTTCACGCGGAGACTGGAACCTGGGAG  
TGTGGCCGGCTGTCCTACTGCTTGAAGACACTGCAGGTGCCCGAGAGAGCCTGCCT  
GCCCTGGCAGAGGGAGGGAAACACTGCAGTTATGGGAGGAAGGGAGCTACGCCAGGA  
TATGCAGGTTCTGGGATGGCAGGGCAGGAAGATGGAATGGTGGAAACAAGATATTGG  
TGAGGGATGATTAGATCTTGGTCAGCTTGCTGAGAAGCTGCCCTCCATC→TCTGTTAC  
CATCCACAGGTGGCTTCCAGCAACTTCTACTGGAGCCCATGCTGAAATTCCACTACAT  
GCTGAAGAAGCTGCAGCTGCATGAGGAGGAGTATGTGCTGATGCAGGCCATCTCCCT  
CTTCTCCCCAGGTGAGGATCTCCCCTAGGCTGCCTGACATCCCCCCCAGCCTTATCTG  
CCCTCCCAGGGAAGGTCCCAGTC

## EXON 9

GAGCAATGCCCTGACTCTGGGCTGGACTGAGCTTGTCTTTGCCCATGATCTTGCACCA  
CACCTCCCTCCCCTCCAGACCGCCAGGTGTGCTGCAGCACCGCGTGGTGG

EPO-Munich  
60  
10. Sep. 1999

## SEQUENCE LISTING

&lt;110&gt; EPIDAUROS Biotechnologie Aktiengesellschaft

<120> Polymorphisms in the human CYP3A4, CYP3A7 and hPXR  
genes and their use in diagnostic and therapeutic  
applications

&lt;130&gt; D2145EP

&lt;140&gt;

&lt;141&gt;

&lt;160&gt; 135

&lt;170&gt; PatentIn Ver. 2.1

&lt;210&gt; 1

&lt;211&gt; 20

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Description of Artificial Sequence: DNA

&lt;400&gt; 1

taatacgact cactataggg

20

&lt;210&gt; 2

&lt;211&gt; 18

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Description of Artificial Sequence: DNA

&lt;400&gt; 2

gaaccattc acatggac

18

&lt;210&gt; 3

&lt;211&gt; 18

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Description of Artificial Sequence: DNA

&lt;400&gt; 3

tgatcatgtc aggatctg

18

&lt;210&gt; 4

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<400> 8  
gaaatctgag gcgggaag 18

<210> 9  
<211> 18  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 9  
gggtcttgtg gattgttg 18

<210> 10  
<211> 18  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 10  
caacaatcca caagaccc 18

<210> 11  
<211> 18  
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<220>  
<223> Description of Artificial Sequence: DNA

<400> 11  
gtgtatcttc gaggcgac 18

<210> 12  
<211> 18  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 12  
ctttccattc ctcaccc 18

<210> 13

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<400> 17  
gagagcaaac ctcatgcc 18

<210> 18  
<211> 18  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 18  
ggcatgaggt ttgctctc 18

<210> 19  
<211> 18  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 19  
ggtgccatcc cttgactc 18

<210> 20  
<211> 18  
<212> DNA  
<213> Artificial Sequence

<220>  
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<400> 20  
gcagaggtgt gggccctg 18

<210> 21  
<211> 23  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 21  
ggagatcaag gaccacgctt gtg 23

<210> 22

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<400> 26  
gcgctgagac tgcctctgt g 21

<210> 27  
<211> 24  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 27  
agtctggctt cctgggttg gctc 24

<210> 28  
<211> 26  
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<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 28  
gaagtggacg tggaaccttc ctggac 26

<210> 29  
<211> 20  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 29  
ctacaaccat ggagacctcc 20

<210> 30  
<211> 20  
<212> DNA  
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<220>  
<223> Description of Artificial Sequence: DNA

<400> 30  
tacctgtccc caccagattc 20

<210> 31



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<400> 35  
ccagtatgag ttgttctctg g 21

<210> 36  
<211> 24  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 36  
aggcagaata tgcttgaacc aggc 24

<210> 37  
<211> 18  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 37  
tcatgtccga cgaggccg 18

<210> 38  
<211> 18  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 38  
cccacatggc tgacatgt 18

<210> 39  
<211> 18  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 39  
cccacatcgagg accagatc 18

<210> 40

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<400> 44  
gctcttggca gtgtccat

18

<210> 45  
<211> 18  
<212> DNA  
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<400> 45  
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18

<210> 46  
<211> 18  
<212> DNA  
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18

<210> 47  
<211> 18  
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<400> 47  
ccttgcatcc ttcacatg

18

<210> 48  
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<220>  
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<400> 48  
catgccgctc tccaggca

18

<210> 49

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<400> 53  
caaggacagc agcatgacag tcac

24

<210> 54  
<211> 20  
<212> DNA  
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<220>  
<223> Description of Artificial Sequence: DNA

<400> 54  
agccaactca gccgcagc

18

<210> 55  
<211> 12  
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<220>  
<223> Description of Artificial Sequence: DNA

<400> 55  
aagcaggatat gg

12

<210> 56  
<211> 12  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 56  
aaaccagtga gt

12

<210> 57  
<211> 12  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 57  
ttctagtcca ag

12

<210> 58

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<400> 62  
ttccgggtag ga

12

<210> 63  
<211> 12  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 63  
tcctagctgc ca

12

<210> 64  
<211> 12  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 64  
cttcaggtag ga

12

<210> 65  
<211> 12  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 65  
tgccagggac tt

12

<210> 66  
<211> 12  
<212> DNA  
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<220>  
<223> Description of Artificial Sequence: DNA

<400> 66  
ctgcaggtgc cc

12

<210> 67

17 / 42

<400> 71  
atgcaggttc tt

12

<210> 72  
<211> 20  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 72  
tcaagtgctg gacttgggac

20

<210> 73  
<211> 20  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 73  
cccactatga tgctgacctc

20

<210> 74  
<211> 18  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 74  
ctgaggcctc tacacatc

18

<210> 75  
<211> 18  
<212> DNA  
<213> Artificial Sequence

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<223> Description of Artificial Sequence: DNA

<400> 75  
aggccctgag atgttacc

18

<210> 76

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<400> 80  
gagcaatgcc ctgactct

18

<210> 81  
<211> 18  
<212> DNA  
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<220>  
<223> Description of Artificial Sequence: DNA

<400> 81  
ccctctggcc atgaagtc

18

<210> 82  
<211> 18  
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<220>  
<223> Description of Artificial Sequence: DNA

<400> 82  
tgcttgtgca gcctcaga

18

<210> 83  
<211> 18  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 83  
gctcttggca gtgtccat

18

<210> 84  
<211> 15  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 84  
tcccagggt tttgt

15

<210> 85

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<400> 89  
aagagagaaa gat

13

<210> 90  
<211> 14  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 90  
tatcttgctc tctt

14

<210> 91  
<211> 14  
<212> DNA  
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<400> 91  
aagagagcaa gata

14

<210> 92  
<211> 13  
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<400> 92  
tactggacag agc

13

<210> 93  
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<220>  
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<400> 93  
gctctgtcca gta

13

<210> 94

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cctgaaaaag gcag

14

<210> 99  
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<212> DNA  
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<400> 99  
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14

<210> 100  
<211> 13  
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<220>  
<223> Description of Artificial Sequence: DNA

<400> 100  
cagtggcggg aaa

13

<210> 101  
<211> 13  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 101  
tttcccggca ctg

13

<210> 102  
<211> 13  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: DNA

<400> 102  
cagtgggtggg aaa

13

<210> 103



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<400> 107  
gtaacagaat ggagg

15

<210> 108  
<211> 249  
<212> DNA  
<213> Homo sapiens

<220>  
<221> intron  
<222> (1)..(115)

<220>  
<221> exon  
<222> (116)..(168)

<220>  
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<222> (169)..(249)

<220>  
<221> CDS  
<222> (116)..(166)

<400> 108  
cctctaactg ccagcaagtc tgatttcatt ggcttcgact gttttcatcc caattagagg 60  
cagggttaag tacattaaaa ataataatca aatattattt tgtttctcct cccag grc 118  
Xaa  
1

ttt tgt atg ttt gac atg gaa tgt cat aaa aag tat gga aaa gtg tgg 166  
Phe Cys Met Phe Asp Met Glu Cys His Lys Lys Tyr Gly Lys Val Trp  
5 10 15

gggtgagtat tctggaaact tccattggat agacttggtt ctatgatgag tttacccac 226  
tgcacagagg acagtctcag ccc 249

<210> 109  
<211> 17  
<212> PRT  
<213> Homo sapiens

<400> 109  
Xaa Phe Cys Met Phe Asp Met Glu Cys His Lys Lys Tyr Gly Lys Val  
1 5 10 15

Trp

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Arg

<210> 112  
 <211> 236  
 <212> DNA  
 <213> Homo sapiens

<220>  
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<220>  
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<220>  
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 g cct ttt ggt cca gtg gga ttt atg aaa agt gcc atc tct ata gct gag 109  
 Pro Phe Gly Pro Val Gly Phe Met Lys Ser Ala Ile Ser Ile Ala Glu  
 1 5 10 15  
 gat gaa gaa tgg aag aga tta cga tca ttg ctg tct cca acc ttc acc 157  
 Asp Glu Glu Trp Lys Arg Leu Arg Ser Leu Leu Ser Pro Thr Phe Thr  
 20 25 30  
 agt gga aaa ctc aag gag gtatgaaaat aacatgagtt ttaataagaa 205  
 Ser Gly Lys Leu Lys Glu  
 35  
 acttaaagaa tgaatctggt ggggacaggt a 236

<210> 113  
 <211> 38  
 <212> PRT  
 <213> Homo sapiens

<400> 113  
 Pro Phe Gly Pro Val Gly Phe Met Lys Ser Ala Ile Ser Ile Ala Glu  
 1 5 10 15

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<210> 115  
 <211> 49  
 <212> PRT  
 <213> Homo sapiens

<400> 115  
 Val Phe Gly Ala Tyr Ser Met Asp Val Ile Thr Ser Thr Ser Phe Gly  
           1                  5                  10                  15  
 Val Asn Ile Asp Ser Leu Asn Asn Pro Gln Asp Pro Phe Val Glu Asn  
                   20                  25                  30  
 Thr Lys Lys Leu Leu Arg Phe Asp Phe Leu Asp Pro Phe Phe Leu Ser  
           35                  40                  45  
 Ile

<210> 116  
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 <212> DNA  
 <213> Homo sapiens

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 <222> (150)..(240)

<220>  
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 <222> (83)..(148)

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 aaatttctct ttttgcttct ag cac cga gtg gat ttc ctt cag ctg atg att 112  
                                   His Arg Val Asp Phe Leu Gln Leu Met Ile  
                                   1                  5                  10  
 gac tct cag aat tca aaa gaa act gag tcc cac aaa ggtaaccaga 158  
 Asp Ser Gln Asn Ser Lys Glu Thr Glu Ser His Lys  
                   15                  20  
 gtgtttctga gggctacttg tggggcactc agaggggaagg ccttggttctg aaaatgtgca 218

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tgc aaa aaa gat gtt gaa atc aat ggg atg ttt att ccc aaa ggg gtg 261
Cys Lys Lys Asp Val Glu Ile Asn Gly Met Phe Ile Pro Lys Gly Val
35 40 45 50

gtg gtg atg att cca agc tat gtt ctt cat cat gac cca aag tac tgg 309
Val Val Met Ile Pro Ser Tyr Val Leu His His Asp Pro Lys Tyr Trp
55 60 65

asa gag cct gag aag ttc ctc cct gaa aggtaggagg cccctgggaa 356
Xaa Glu Pro Glu Lys Phe Leu Pro Glu
70 75

gggagccctc cctgaaccag cctgggttcaa gcatattctg cct 399

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<210> 119  
 <211> 75  
 <212> PRT  
 <213> Homo sapiens

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<400> 119
Ala Pro Pro Thr Tyr Asp Thr Val Leu Gln Leu Glu Tyr Leu Asp Met
1 5 10 15

Val Val Asn Glu Thr Leu Arg Leu Phe Pro Val Ala Met Arg Leu Glu
20 25 30

Arg Val Cys Lys Lys Asp Val Glu Ile Asn Gly Met Phe Ile Pro Lys
35 40 45

Gly Val Val Val Met Ile Pro Ser Tyr Val Leu His His Asp Pro Lys
50 55 60

Tyr Trp Xaa Glu Pro Glu Lys Phe Leu Pro Glu
65 70 75

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<210> 120  
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 <213> Homo sapiens

<220>  
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 <222> (1)..(66)

<220>  
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<220>  
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 <222> (751)..(894)

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&lt;213&gt; Homo sapiens

&lt;400&gt; 121

Met Thr Val Thr Arg Thr His His Phe Lys Glu Gly Ser Leu Arg Ala  
 1 5 10 15

Pro Ala Ile Pro Leu His Ser Ala Ala Ala Glu Leu Ala Ser Asn  
 20 25 30

&lt;210&gt; 122

&lt;211&gt; 345

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; intron

&lt;222&gt; (1)..(60)

&lt;220&gt;

&lt;221&gt; exon

&lt;222&gt; (61)..(279)

&lt;220&gt;

&lt;221&gt; intron

&lt;222&gt; (280)..(345)

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (62)..(277)

&lt;400&gt; 122

ctgaggcctc tacacatccc tgtccagtct tttcattctc tgtgggttttc tcattttctag 60

t cca aga ggc cca gaa gca aac ctg gag gtg aga ccc aaa gaa agc tgg 109  
 Pro Arg Gly Pro Glu Ala Asn Leu Glu Val Arg Pro Lys Glu Ser Trp  
 1 5 10 15

aac cat gct gac ttt gta cac tgt gag gac aca gag tct gtt cct gga 157  
 Asn His Ala Asp Phe Val His Cys Glu Asp Thr Glu Ser Val Pro Gly  
 20 25 30

aag ccc agt gtc aac gca gat gag gaa gtc gga ggt ccc caa atc tgc 205  
 Lys Pro Ser Val Asn Ala Asp Glu Glu Val Gly Gly Pro Gln Ile Cys  
 35 40 45

cgt gta tgt ggg gac aag gcc act ggc tat cac ttc aat gtc atg aca 253  
 Arg Val Cys Gly Asp Lys Ala Thr Gly Tyr His Phe Asn Val Met Thr  
 50 55 60

tgt gaa gga tgc aag ggc ttt ttc aggtagagtt acccatcagc cttcaccac 307  
 Cys Glu Gly Cys Lys Gly Phe Phe  
 65 70

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```

ccc ctt ccg gaa ggg cgc ctg cga gat cac ccg gaa gac ccg gcg aca 159
Pro Leu Pro Glu Gly Arg Leu Arg Asp His Pro Glu Asp Pro Ala Thr
          15                20                25

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gtg cca ggc ctg ccg cct gcg caa gtg cct gga gag ccg cat gaa gaa 207
Val Pro Gly Leu Pro Pro Ala Gln Val Pro Gly Glu Arg His Glu Glu
          30                35                40

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gga gagtgcgcag tgggcgcgcg ggcgggcccg cgccgggggtg cacggctctg 260
Gly

```

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agtaaggacg tgccgtgggt gtgtgcatgc ttgtgtggag atgcgcgcgc agtgtgcgcg 320

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tgaacacacg tgcacatgtg agct 344

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<211> 44
<212> PRT
<213> Homo sapiens

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Glu Gly His Glu Thr Gln Arg Pro Ala Glu Val Pro Leu Pro Glu Gly
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Arg Leu Arg Asp His Pro Glu Asp Pro Ala Thr Val Pro Gly Leu Pro
          20                25                30

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Pro Ala Gln Val Pro Gly Glu Arg His Glu Glu Gly
          35                40

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<210> 126
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<220>
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&lt;222&gt; (1)..(69)

&lt;220&gt;

&lt;221&gt; exon

&lt;222&gt; (70)..(344)

&lt;220&gt;

&lt;221&gt; intron

&lt;222&gt; (345)..(415)

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (70)..(342)

&lt;400&gt; 128

ctgagttggg acctgtctat gaaagcacat gctgtctctc ctctgtccac ctcttgccat 60

gtgtcctag ctg cca ggg gtg ctt agc agt ggc tgc gag ttg cca gag tct 111  
 Leu Pro Gly Val Leu Ser Ser Gly Cys Glu Leu Pro Glu Ser  
           1                                  5                                  10

ctg cag gcc cca tcg agg gaa gaa gct gcc aag tgg agc cag gtc cgg 159  
 Leu Gln Ala Pro Ser Arg Glu Glu Ala Ala Lys Trp Ser Gln Val Arg  
           15                                  20                                  25                                  30

aaa gat ctg tgc tct ttg aag gtc tct ctg cag ctg cgg ggg gag gat 207  
 Lys Asp Leu Cys Ser Leu Lys Val Ser Leu Gln Leu Arg Gly Glu Asp  
                                   35                                  40                                  45

ggc agt gtc tgg aac tac aaa ccc cca gcc gac agt ggy ggg aaa gag 255  
 Gly Ser Val Trp Asn Tyr Lys Pro Pro Ala Asp Ser Xaa Gly Lys Glu  
                                   50                                  55                                  60

atc ttc tcc ctg ctg ccc cac atg gct gac atg tca acc tac atg ttc 303  
 Ile Phe Ser Leu Leu Pro His Met Ala Asp Met Ser Thr Tyr Met Phe  
                                   65                                  70                                  75

aaa ggc atc atc agc ttt gcc aaa gtc atc tcc tac ttc aggtaggaca 352  
 Lys Gly Ile Ile Ser Phe Ala Lys Val Ile Ser Tyr Phe  
           80                                  85                                  90

tggagactgg gtggttgggt gtggaaaaga actggaagtg gccaggaggt tcaaagggcc 412

tgg 415

&lt;210&gt; 129

&lt;211&gt; 91

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 129

Leu Pro Gly Val Leu Ser Ser Gly Cys Glu Leu Pro Glu Ser Leu Gln  
           1                                  5                                  10                                  15

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tgccagg gac ttg ccc atc gag gac cag atc tcc ctg ctg aag ggg gcc 109  
 Asp Leu Pro Ile Glu Asp Gln Ile Ser Leu Leu Lys Gly Ala  
 1 5 10

gct ttc gag ctg tgt caa ctg aga ttc aac aca gtg ttc aac gcg gag 157  
 Ala Phe Glu Leu Cys Gln Leu Arg Phe Asn Thr Val Phe Asn Ala Glu  
 15 20 25 30

act gga acc tgg gag tgt ggc cgg ctg tcc tac tgc ttg gaa gac act 205  
 Thr Gly Thr Trp Glu Cys Gly Arg Leu Ser Tyr Cys Leu Glu Asp Thr  
 35 40 45

gca ggt gcccgagaga gcctgcctgc cctggcagag ggagggaaac actgcagtta 261  
 Ala

tgggaggaag ggagctacgc caggatatgc aggttctggg atggcagggc aggaagatgg 321

aatggtggaa aacaagatat tggtgagggg tgattagatc ttggtcagct tgctgagaag 381

ctgccccctcc atyctgttac catccacagg t ggc ttc cag caa ctt cta ctg 433  
 Gly Phe Gln Gln Leu Leu Leu  
 50 55

gag ccc atg ctg aaa ttc cac tac atg ctg aag aag ctg cag ctg cat 481  
 Glu Pro Met Leu Lys Phe His Tyr Met Leu Lys Lys Leu Gln Leu His  
 60 65 70

gag gag gag tat gtg ctg atg cag gcc atc tcc ctc ttc tcc cca 526  
 Glu Glu Glu Tyr Val Leu Met Gln Ala Ile Ser Leu Phe Ser Pro  
 75 80 85

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aaggtcccag tc 598

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<211> 86

<212> PRT

<213> Homo sapiens

<400> 131

Asp Leu Pro Ile Glu Asp Gln Ile Ser Leu Leu Lys Gly Ala Ala Phe  
 1 5 10 15

Glu Leu Cys Gln Leu Arg Phe Asn Thr Val Phe Asn Ala Glu Thr Gly  
 20 25 30

Thr Trp Glu Cys Gly Arg Leu Ser Tyr Cys Leu Glu Asp Thr Ala Gly  
 35 40 45

Gly Phe Gln Gln Leu Leu Leu Glu Pro Met Leu Lys Phe His Tyr Met  
 50 55 60



1                      5                      10                      15  
Phe Ala Ile Thr Leu Lys Ser Tyr Ile Glu Cys Asn Arg Pro Gln Pro  
                20                      25                      30

Ala His

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tcttttctct ggctggcatg cagg ttc ttg ttc ctg aag atc atg gct atg 111  
Phe Leu Phe Leu Lys Ile Met Ala Met  
1 5

ctc acc gag ctc cgc agc atc aat gct cag cac acc cag cgg ctg ctg 159  
Leu Thr Glu Leu Arg Ser Ile Asn Ala Gln His Thr Gln Arg Leu Leu  
10 15 20 25

cgc atc cag gac ata cac ccc ttt gct acg ccc ctg atg cag gag ttg    207  
Arg Ile Gln Asp Ile His Pro Phe Ala Thr Pro Leu Met Gln Glu Leu  
                30                       35                            40

ttc ggc atc aca ggt agc tgagcggctg cccttgggtg acacctccga 255  
Phe Gly Ile Thr Gly Ser  
45

gagggagcca gacccagagc cctctgagcc gccactcccg ggccaagaca gatggacact 315  
gccaagagc 324

10. Sep. 1999

**Abstract**

Described are general means and methods of diagnosing and treating the phenotypic spectrum as well as the overlapping clinical characteristics with several forms of inherited abnormal expression and/or function of the CYP3A4, CYP3A7 and hPXR genes. In particular, polynucleotides of molecular variant CYP3A4, CYP3A7 and hPXR genes which, for example, are associated with insufficient metabolism and/or sensitivity of drugs, and vectors comprising such polynucleotides are provided. Furthermore, host cells comprising such polynucleotides or vectors and their use for the production of variant CYP3A4, CYP3A7 and hPXR proteins are described. In addition, variant CYP3A4, CYP3A7 and hPXR proteins and antibodies specifically recognizing such proteins as well as transgenic non-human animals comprising the above-described polynucleotide or vectors are provided. Described are also methods for identifying and obtaining inhibitors for therapy of disorders related to the malfunction of the CYP3A4, CYP3A7 and hPXR genes as well as methods of diagnosing the status of such disorders. Pharmaceutical and diagnostic compositions comprising the above-described polynucleotides, vectors, proteins, antibodies and inhibitors by the above-described method are provided. Said compositions are particularly useful for diagnosing and treating various diseases with drugs that are substrates, inhibitors or modulators of the CYP3A4, CYP3A7 or hPXR gene product.